Reaction of sulphate radical anion ($SO_4^{\bullet-}$) with hydroxy- and methyl-substituted pyrimidines: a pulse radiolysis study

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Abstract—Reactions of sulphate radical anion ($SO_4^{\bullet-}$) with 4,6-dihydroxy-2-methyl pyrimidine (DHMP), 2,4-dimethyl-6-hydroxy pyrimidine (DMHP), 6-methyl uracil (MU) and 5,6-dimethyl uracil (DMU) have been studied by pulse radiolysis at pH 3 and at pH 10. The transient intermediate spectra were compared with those from the reaction of hydroxyl radical ($\cdot$OH). It is proposed that $SO_4^{\bullet-}$ produces radical cations of these pyrimidines in the initial stage. These radical cations are short-lived except in the case of DMHP where a relatively longer lived radical cation is proposed to be formed. When there is a hydrogen atom attached to the N(1) or N(3) position, a deprotonation from these sites is highly favored. When there is no hydrogen attached to these sites, deprotonation from a substituted methyl group is favored. At acidic pH, deprotonation from nitrogen is observed for DHMP, MU and DMU. At basic pH, the radical cation reacts with OH^- leading to the formation of OH adducts.

Keywords: Pulse radiolysis; sulphate radical anion; deprotonation; transient intermediate spectra.

INTRODUCTION

The deleterious effects of ionizing radiation in living cells are considered to be mainly due to alterations of their DNA [1]. It is known that DNA damage could be caused either by direct effect or by indirect effect. Direct effect is by the absorption of the radiation energy directly by DNA itself. Such an effect could give rise to the formation of DNA radical cations and electrons [1]. A radical cation of nucleobases produced by radiolysis in a freon matrix has already been reported [2]. The indirect effect is due to the absorption of energy by the water surrounding the DNA and the

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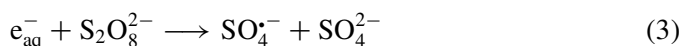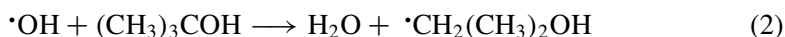*To whom correspondence should be addressed. Fax: (91-481) 273-1009; e-mail: mgu@vsnl.com
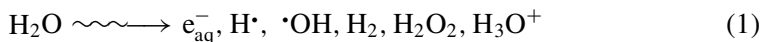
subsequent reaction of water radicals with DNA. Dilute aqueous solutions always favor the indirect effect [2]. On the other hand, the direct effect can be studied in aqueous solution by conveniently producing radical cations of the nucleobases using photoionization [3–8], photosensitization with quinones [9–14], Tl^{2+} [15, 16], $Br_2^{\bullet-}$ [16–19] and $SO_4^{\bullet-}$ [16, 20–37]. Therefore, the reaction of $SO_4^{\bullet-}$ has the advantage in generating base radical cations thereby creating the direct effect on a model level in aqueous medium.
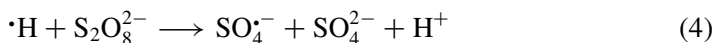
The radical cations of nucleobases are generally short-lived, generated from a still very short-lived sulphate radical adduct in aqueous solution [37]. Although the base radical cations are short-lived, there are sufficient evidences for their formation available such as in the case of thymine derivatives [36]. The subsequent reactions of the radical cation depends mainly on the substituents and their positions in the pyrimidine ring. For example, a N-centered radical (at the N(1) position) is formed from the radical cation of uracil [20], while an OH adduct is formed when the N(1) position is substituted by a methyl group [1]. It is also reported that an allyl type radical can be formed when there is a methyl substituent at the pyrimidine ring [38]. In order to investigate the substituent effects in detail, pulse radiolysis has been carried out with 2,4-dimethyl-6-hydroxy pyrimidine (DMHP), 4,6-dihydroxy-2-methyl pyrimidine (DHMP), 5,6-dimethyl uracil (DMU) and 6-methyl uracil (MU) at acidic and basic pH, and the results obtained with these substrates are presented in this paper. The properties of the transient intermediates have been compared with those obtained from the reactions of hydroxyl radicals as one of the probable reactions of the base radical cations in aqueous medium is its reaction with water leading to the formation of OH adduct radicals.

EXPERIMENTAL

Commercially available DHMP, DMHP, MU and DMU and potassium peroxodisulphate ($K_2S_2O_8$) (Aldrich) and 2-methyl propan-2-ol (SRL) were used without further purification. The solutions were prepared in water which had been purified using a Millipore–Milli-Q system. The concentrations of the substrates were maintained at 1×10^{-3} mol dm^{-3}, 2-methyl propan 2-ol at 0.2 mol dm^{-3} and $K_2S_2O_8$ at 10^{-2} mol dm^{-3}. The solutions were saturated with N_2 before irradiation for removing O_2.

$SO_4^{\bullet-}$ can be produced from the reaction of e_{aq}^- and $H^\bullet$ with $S_2O_8^{2-}$:

$$H_2O \rightsquigarrow e_{aq}^-, H^\bullet, {}^\bullet OH, H_2, H_2O_2, H_3O^+ \tag{1}$$

$$ {}^\bullet OH + (CH_3)_3COH \longrightarrow H_2O + {}^\bullet CH_2(CH_3)_2OH \tag{2}$$

$$ e_{aq}^- + S_2O_8^{2-} \longrightarrow SO_4^{\bullet-} + SO_4^{2-} \tag{3}$$

$$\cdot H + S_2O_8^{2-} \longrightarrow SO_4^{\cdot-} + SO_4^{2-} + H^+ \tag{4}$$

Under these conditions the yield $G(SO_4^{\cdot-}) \approx 3.4 \times 10^{-7}$ mol J^{-1} [39, 40].

N_2O-saturated aqueous solutions, typically containing 10^{-3} mol dm^{-3} of the substrate, were pulse irradiated using a linear accelerator delivering 7 MeV electron pulses of 50 ns duration. KSCN dosimetry was used to determine the dose per pulse using $G \times \varepsilon_{(500\ nm)} = 21\,520$ dm^3 mol^{-1} cm^{-1} in aerated solutions and the dose per pulse was normally kept at 10 Gy. The details of the pulse radiolysis set-up have been described elsewhere [41, 42].

RESULTS AND DISCUSSION

4,6-Dihydroxy-2-methyl pyrimidine (DHMP)

The time-resolved absorption spectra obtained with DHMP at pH 3.0 have two distinct peaks at 340 and 610 nm (Fig. 1). These spectra were appeared to undergo a second order decay. A typical trace obtained at 340 nm is also shown in Fig. 1. The time-resolved spectra are also recorded at pH 10 (Fig. 2). The spectrum obtained
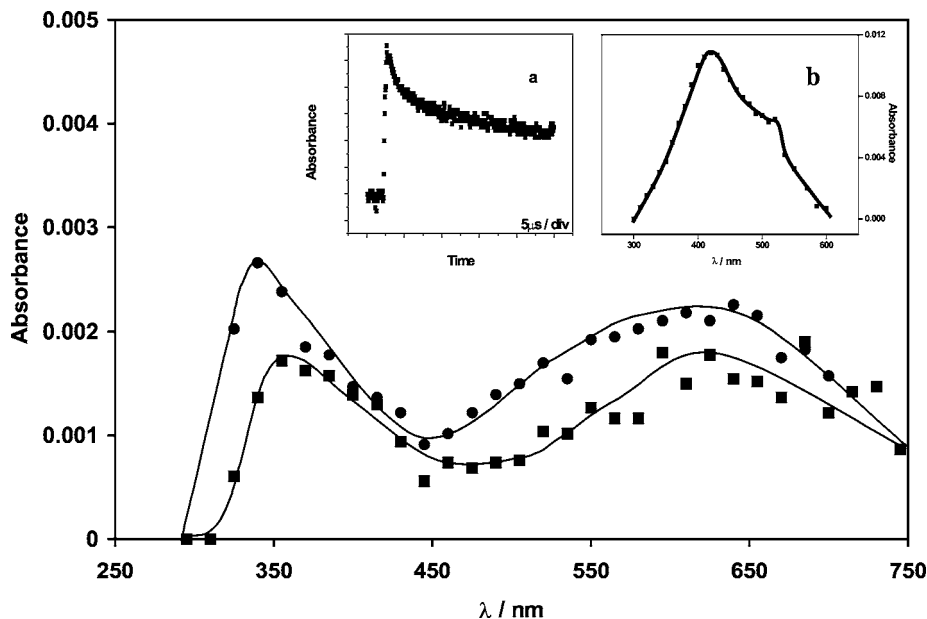

Figure 1. Transient absorption spectra (calculated for 10 Gy/pulse) obtained from the radiolysis of a N_2-saturated aqueous solution of DHMP (1×10^{-3} mol dm^{-3}) containing $K_2S_2O_8$ (0.2 mol dm^{-3}) and 2-methyl propan-2-ol (0.2 mol dm^{-3}) at pH 3.0 at 3 μs (●) and 40 μs (■) after the pulse. Inset: (a) Decay trace of the intermediate obtained at 340 nm. (b) The OH adduct spectrum of DHMP at pH 4.5 at 1 μs after the pulse [43] (the absorbance value is calculated for a typical dose rate of 10 Gy/pulse and a $G(\cdot OH)$ of 2.7×10^{-7} mol J^{-1}).

at 2 μs after the pulse has two absorption maxima around 300 and 360 nm and has a broad maximum around 600 nm. After about 40 μs, the broad absorption around 600 nm region has fully disappeared and the spectrum has a well-defined peak at 300 nm. The absorption build-up at 300 nm is shown in the inset of Fig. 2.

$SO_4^{\bullet-}$ is known to add to the pyrimidine ring followed by the elimination of SO_4^{2-} leading to the formation of a radical cation [36]. It is further known that both the sulphate radical adducts, as well as the radical cations, are short-lived and that these are difficult to be detected in the microsecond time scale, though there are some indications of the absorbance due to the radical cations in less than a microsecond [36]. Therefore, in a most reasonable assumption, in the present case, the probable radical products resulting from a radical cation of DHMP have been considered. These include (i) the OH adduct radicals (C(5)-yl or C(6)-yl) resulting from the hydrolysis of the radical cation, (ii) an N-centered radical resulting from the deprotonation of the radical cation and (iii) an allylic radical resulting from the deprotonation of the radical cation from the methyl group. The formation of the OH adducts, as per possibility (i) above, is a highly probable reaction, observed with several substituted pyrimidines [36]. In order to look at this possibility at pH 3 in the present case, an OH adduct spectrum obtained with DHMP at pH 4.5 is compared in Fig. 1 [43]. It is clearly seen that this OH adduct spectrum is very different from the radical spectra obtained with $SO_4^{\bullet-}$ at pH 3. Therefore, possibility (i) can be fully ruled out. Although, the formation of allylic radical (possibility (iii)) is possible according to earlier reports [34, 35, 38], the deprotonation normally takes place from the nitrogen when a H atom is attached to N(1) or N(3) [38]. This leads to the consideration of the probability of the formation of a neutral N-centered radical of DHMP which could be in resonance with an oxyradical. A study by Novais and Steenken [21] reported EPR evidence for the formation of an oxyradical and its deprotonated form (at pH $>$ 5) in dihydroxy pyrimidine systems. Therefore, it is proposed that the species at pH 3 with the absorption spectra having maxima at 340 and 610 nm is the neutral oxyradical of DHMP (Scheme 1).

There is a high probability for the formation of OH adduct radicals at higher pH from the reaction of OH^- with the radical cation [36]. In Fig. 2, an OH adduct radical spectra of DHMP recorded 40 μs after the pulse at pH 10.4 is compared with the spectra obtained with $SO_4^{\bullet-}$ at pH 10. In a recent study [43] we have demonstrated the formation of a deprotonated OH adduct of DHMP at pH 10.4 (as DHMP is already in the deprotonated form at basic pH with pK value 6.35). This

(pH 3)
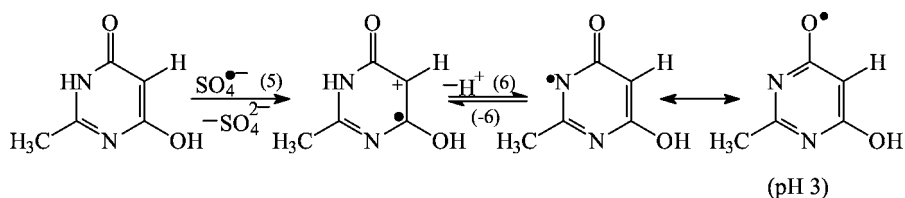
Scheme 1. Proposed mechanism of the reaction of sulphate radical with DHMP.

negatively charged OH adduct has three absorption maxima at around 290, 390 and 480 nm which undergoes a fast decay at $\lambda > 350$ nm and gets transformed into a new species with a spectral maxima at 300 nm [43]. It is indeed the same spectral behavior in the present case too. The initial spectrum (2 μs) has λ_{max} at 300 and 360 nm and a broad absorption at $\lambda > 500$ nm which undergoes a fast decay at higher wavelengths and gets transformed into a species with spectral maxima at 300 and 360 nm. Similar to the case of OH reaction [43] the absorption trace at 300 nm in the present case has also shown a clear absorption build up as a second step (see Fig. 2, inset). It is, therefore, explained as the formation of a deprotonated OH adduct resulting from the reaction of the radical cation with OH^- at pH 10 and it undergoes a dehydration reaction forming the corresponding oxy radical as shown in Scheme 2. Such a dehydration reaction is clearly demonstrated in our recent study [43]. The absorption build up at 300 nm (Fig. 2) is therefore attributed to the dehydration reaction ($k = 8 \times 10^4$ s^{-1}).
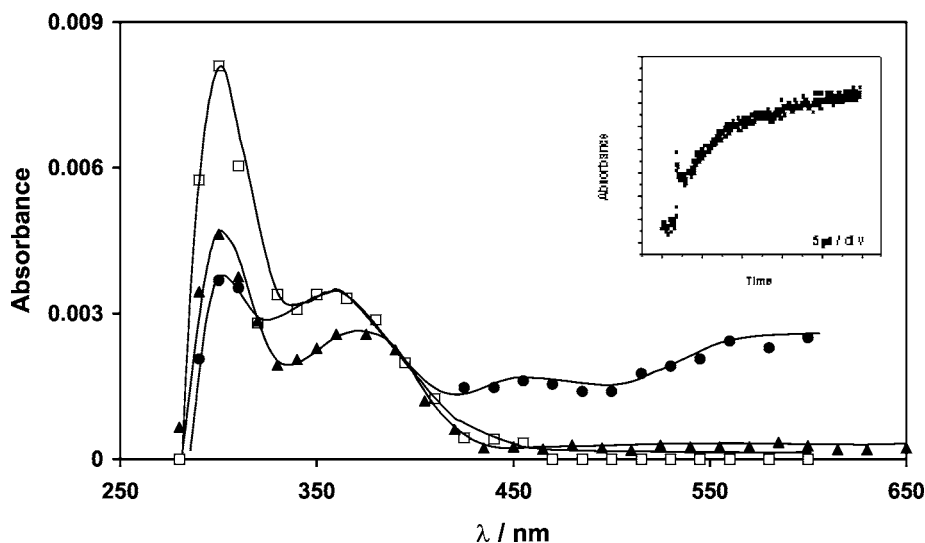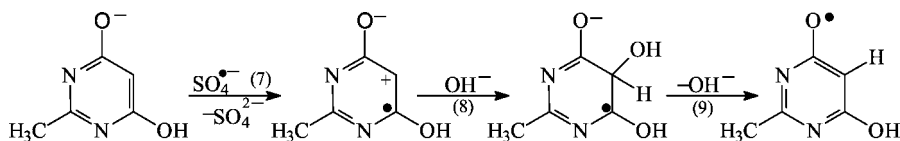

Figure 2. Transient absorption spectra obtained from the radiolysis of a N_2-saturated aqueous solution of DHMP (1×10^{-3} mol dm^{-3}) containing $K_2S_2O_8$ (0.2 mol dm^{-3}) and 2-methyl propan-2-ol (0.2 mol dm^{-3}) at pH 9.9 at 2 μs ($\bullet$) and 40 μs ($\square$) after the pulse, and the OH adduct spectrum of DHMP [43] at pH 10.4 at 40 μs after the pulse ($\blacktriangle$). The absorbance is calculated for a typical dose rate of 10 Gy/pulse and a $G(^{\cdot}OH)$ of 2.7×10^{-7} mol J^{-1}. Inset: the intermediate trace obtained at 300 nm from the reaction of $SO_4^{\cdot-}$.

Scheme 2. Proposed mechanism of the reaction of sulphate radical with DHMP at pH 10.

2,4-Dimethyl-6-hydroxy pyrimidine (DMHP)

The transient absorption spectrum obtained at 3 μs after the pulse at pH 3 has a distinct absorption maximum at 370 nm with a shoulder around 300 nm and a broad maximum around 470 nm (Fig. 3). The OH adduct spectrum of DMHP at pH 6 with two absorption maxima at 325 and 450 nm [43] has no comparable features with that obtained with $SO_4^{\bullet-}$. However, when the spectrum obtained from the reaction of $O^{\bullet-}$ with DMHP at pH > 13 is compared with this spectrum [44], a high degree of similarity in the spectral features were observed (see Fig. 3). At pH 10, the transient spectrum obtained at 3 μs after the pulse, has a λ_{max} at 380 nm with a shoulder at 330 nm. However, the spectrum recorded after 40 μs showed a clear transformation from the initial spectrum with two peaks at 330 and 430 nm. This transformed spectrum is very similar to the OH adduct spectrum of DMHP at pH 10 (Fig. 4).

It can be seen from Fig. 3 that the initial species is quite unstable and it undergoes a fast decay at 370 nm and above 580 nm. However the decay of the spectra at 300 and around 470 nm appears to be very slow. Assuming that the absorbance around 370 nm belongs to the contribution of a still undecayed initial spectrum, the transformed species has similarities with the spectrum obtained from the $O^{\bullet-}$ reaction. From these spectral comparisons, it is proposed that the initial decay observed at 370 nm is due to the decay of the radical cation of DMHP which on
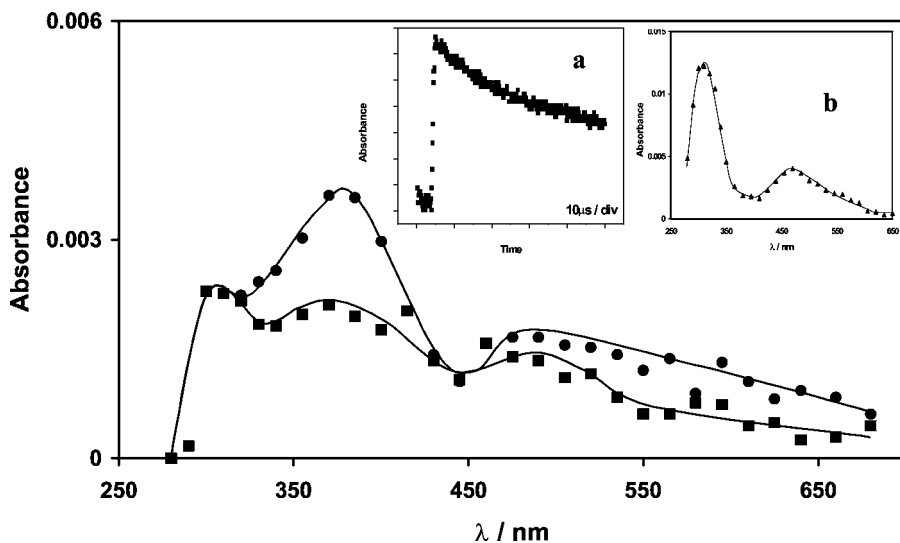

Figure 3. Transient absorption spectra (calculated for 10 Gy/pulse) obtained from the radiolysis of a N_2-saturated aqueous solution of DMHP (1×10^{-3} mol dm^{-3}) containing $K_2S_2O_8$ (0.2 mol dm^{-3}) and 2-methyl propan-2-ol (0.2 mol dm^{-3}) at pH 2.6 at 3 μs ($\bullet$) and 40 μs ($\blacksquare$) after the pulse. Inset: (a) decay trace of the intermediate at 370 nm. (b) The transient spectrum obtained from the reaction of $O^{\bullet-}$ with DMHP [44] at pH 13 at 6 μs after the pulse. The absorbance is calculated for a typical dose rate of 10 Gy/pulse and a $G(O^{\bullet-})$ of 2.7×10^{-7} mol J^{-1}.

deprotonation from the methyl group gives rise to an allylic radical as shown in Scheme 3. It must be, therefore, assumed that the radical cation is stable in the microsecond time scale, though no clear evidence is available at this point. Such longer-lived radical cations were reported in the case of dimethyl and trimethyl substituted (at the nitrogens) thymines [36]. Furthermore, the relative stability of this radical cation compared to that of DHMP is attributed to electron-donating nature of the methyl group (-I effect). As DMHP has no H atom bonded to nitrogen (it exists as its enol form, see its structure) and it has two substituted methyl groups, the probability of H^+ elimination from the methyl group is quite conceivable. The
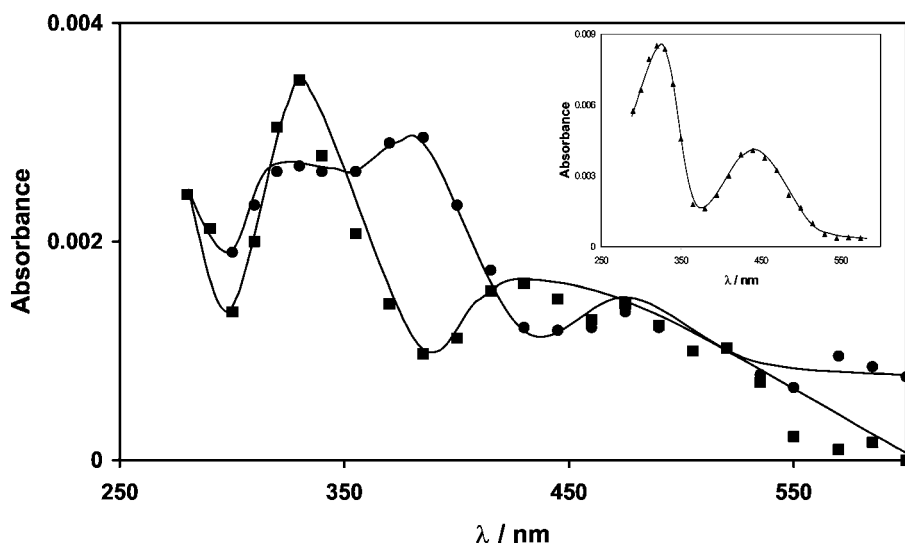

Figure 4. Transient absorption spectra (calculated for 10 Gy/pulse) obtained from the radiolysis of a N_2-saturated aqueous solution of DMHP (1×10^{-3} mol dm^{-3}) containing $K_2S_2O_8$ (0.2 mol dm^{-3}) and 2-methyl propan-2-ol (0.2 mol dm^{-3}) at pH 10 at 3 μs (●) and 40 μs (■). Inset: OH-adduct spectrum of DMHP at pH 10.4 at 3 μs after the pulse [43]. The absorbance is calculated for a typical dose rate of 10 Gy/pulse and a $G(\cdot OH)$ of 2.7×10^{-7} mol J^{-1}.
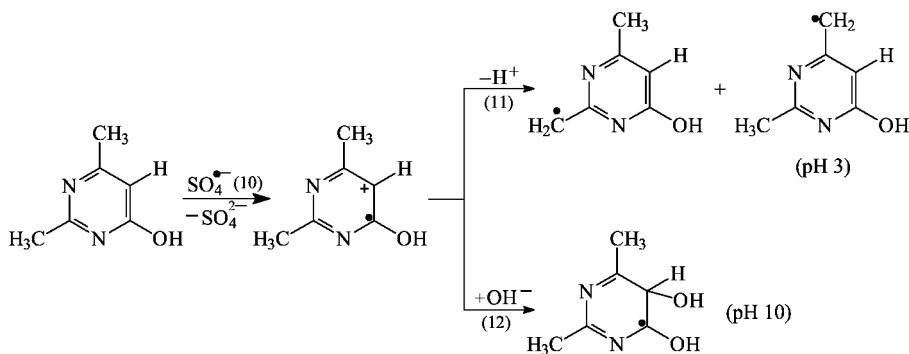
Scheme 3. Proposed mechanism of the reaction of sulphate radical with DMHP.

similarity of this spectrum with the spectra obtained from the reaction of $O^{\bullet-}$ with DMHP (see Fig. 3) where an allylic radical is reported [44], gives a clear support to this interpretation. As shown in Scheme 3, two types of allylic radicals could be formed due to the abstraction of hydrogen from C(2) and C(4) methyl groups. Therefore, the observed transient spectrum is due to the combined effect of both these radicals or to the one which is formed high in yield. However, a clear distinction could not be made between these two radical spectra [44]. In a recent report we have demonstrated the deprotonation of the radical cation of $2'$-deoxycytidine from the $-NH_2$ group leading to the formation of a N-centered radical at neutral pH [45]. At higher pH, the decay of the initially formed radical cation is faster at around 370 nm and the resulting radical which has absorption maxima at 330 and 430 nm is proposed as the OH adduct of DMHP. This is very clear from the close similarities of the spectrum recorded at 40 μs after the pulse in the case of the reaction of $SO_4^{\bullet-}$ and of $^{\bullet}OH$ (Fig. 4). The OH adduct spectrum of DMHP is attributed to the neutral C(6)-ylC(5)OH radical [43]. The possibility for the formation of OH adduct from the reaction of the initially formed radical cation of DMHP (whose absorption decay is visible at 370 nm, Fig. 4) is quite conceivable as at higher concentrations of OH^-, its reaction with the radical cation can effectively compete with the deprotonation from the methyl groups (Scheme 3). As DMHP has a pK value at 9.9, almost about 50% exists in its anionic form. However, it is reported that the OH adduct of DMHP has a pK value well above 10.5 [43]. Therefore, the proposed OH adduct at pH 10 exists in its neutral form.

6-Methyl uracil (MU) and 5,6-dimethyl uracil (DMU)

The transient spectrum obtained for MU at pH 3.0 showed a λ_{max} at 440 nm and a shoulder around 300 nm (Fig. 5). The OH adduct spectrum obtained with MU was, however, different from these spectra (Fig. 5). The transient spectra at pH 9.5 have very similar properties like the OH adduct spectra (Fig. 6).

   The intermediate radicals produced in uracil systems are relatively well documented [1, 36–38]. The deprotonation from the NH group of the initially formed radical cation is a highly favored reaction [37]. It is therefore proposed that a N-centered radical intermediate is formed from the deprotonation of the radical cation of MU and the intermediate spectrum is attributed to this N-centered radical (Scheme 4). The assignment of this spectrum to the N-centered radical is undoubtedly proposed based on the EPR evidence reported by Novais and Steenken [21]. The spectra obtained at higher pH values gave a clear indication for the existence of the OH adduct radicals as the OH adduct spectrum recorded at 40 μs (at pH 10.4) has very similar features with that of the spectra obtained from the reaction of $SO_4^{\bullet-}$ at pH 9.5 (see Fig. 6). It is reported that the initially formed deprotonated OH adduct (MU has a pK value at 9.8) undergoes a base-catalyzed transformation reaction to an oxy radical in alkaline medium [43]. It is clear from these observations that the initially formed radical cation of MU can effectively react with OH^- in competition with the deprotonation at the nitrogen at alkaline pH values and hence an OH
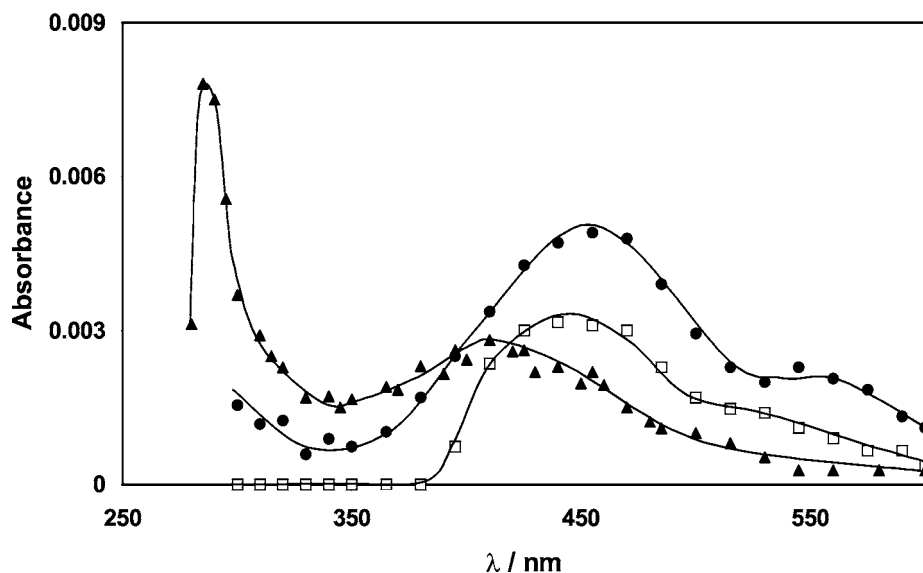
Figure 5. Transient absorption spectra (calculated for 10 Gy/pulse) obtained from the radiolysis of a N$_2$-saturated aqueous solution of MU (1×10^{-3} mol dm^{-3}) containing K$_2$S$_2$O$_8$ (0.2 mol dm^{-3}) and 2-methyl propan-2-ol (0.2 mol dm^{-3}) at 2 μs (●) and 40 μs (□) after the pulse at pH 3.0, and the OH adduct spectrum of MU at pH 6 at 2 μs after the pulse (▲) [43]. The absorbance is calculated for a typical dose rate of 10 Gy/pulse and a G(•OH) of 2.7×10^{-7} mol J^{-1}.
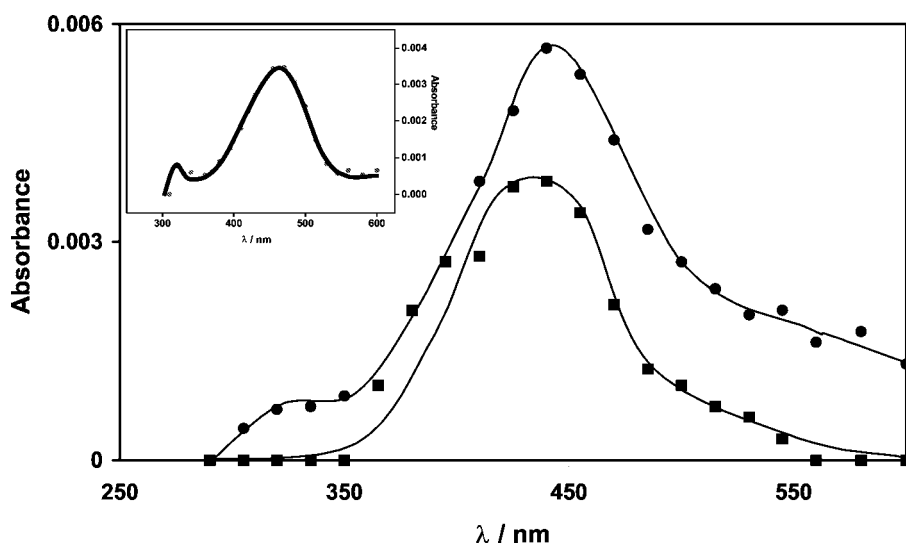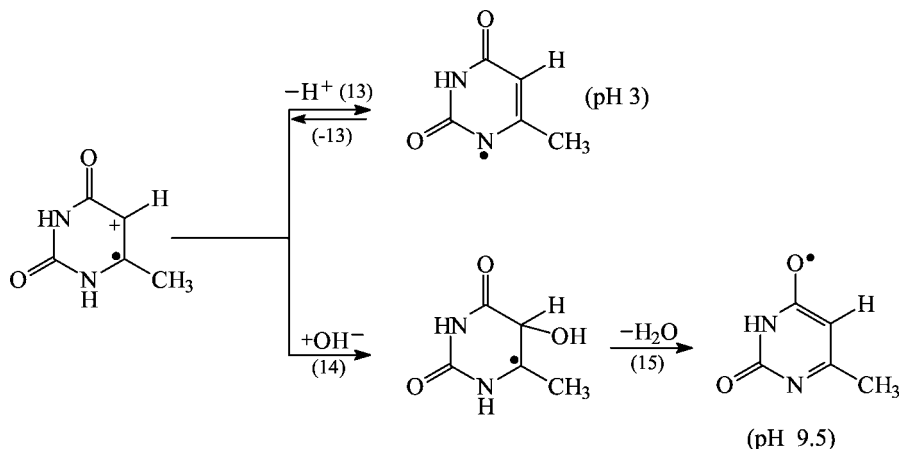

Figure 6. Transient absorption spectra (calculated for 10 Gy/pulse) obtained from the radiolysis of a N$_2$-saturated aqueous solution of MU (1×10^{-3} mol dm^{-3}) containing K$_2$S$_2$O$_8$ (0.2 mol dm^{-3}) and 2-methyl propan-2-ol (0.2 mol dm^{-3}) at 2 μs (●) and 40 μs (■) after the pulse at pH 9.5. Inset: the OH adduct spectrum of MU at pH 40 μs after the pulse at pH 10.4 [43]. The absorbance is calculated for a typical dose rate of 10 Gy/pulse and a G(•OH) of 2.7×10^{-7} mol J^{-1}.

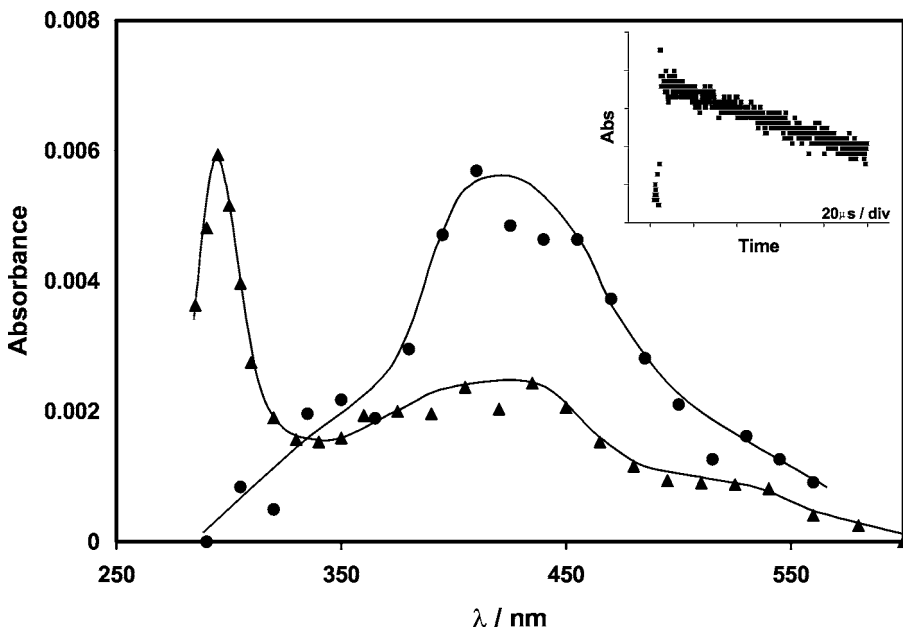**Scheme 4.** Proposed mechanism of the reaction of sulphate radical with MU.

Figure 7. Transient absorption spectrum (calculated for 10 Gy/pulse) obtained from the radiolysis of a N$_2$-saturated aqueous solution of DMU (1 × 10^{-3} mol dm^{-3}) containing K$_2$S$_2$O$_8$ (0.2 mol dm^{-3}) and 2-methyl propan-2-ol (0.2 mol dm^{-3}) at 2 μs after the pulse at pH 3.0 (●) and the OH adduct spectrum of DMU at pH 6 at 1 μs after the pulse (▲) [43]. The absorbance is calculated for a typical dose rate of 10 Gy/pulse and a G(·OH) of 2.7 × 10^{-7} mol J^{-1}. Inset: decay trace of the intermediates at 400 nm from the reaction of SO$_4^{\bullet-}$.

adult of MU could be resulted. However, this OH adduct can, in turn, undergo a base elimination reaction to form an oxyradical of MU as shown in Scheme 4. Therefore, the observed transient spectra are attributed to the oxyradical of MU. As
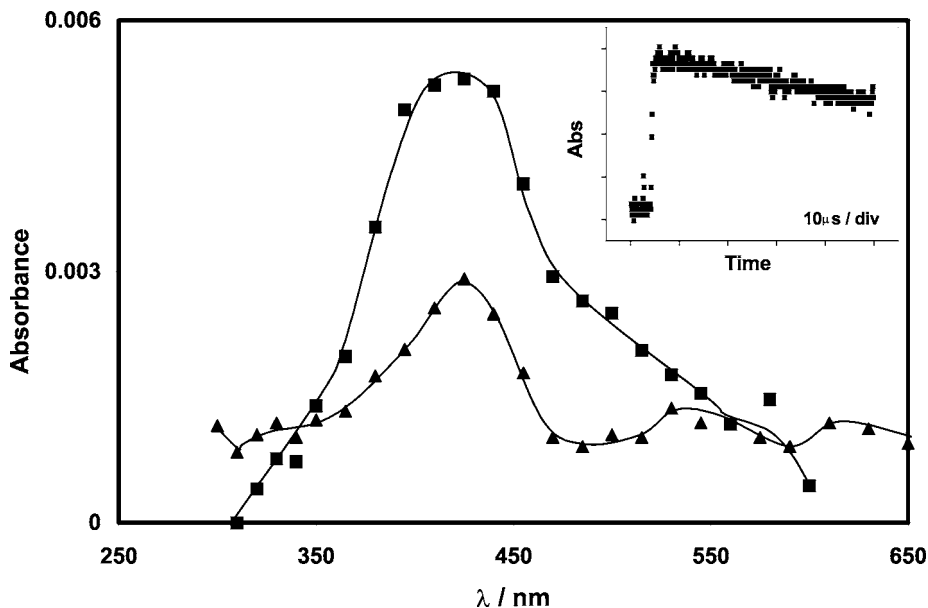
Figure 8. Transient absorption spectrum (calculated for 10 Gy/pulse) obtained from the radiolysis of a N$_2$-saturated aqueous solution of DMU (1×10^{-3} mol dm^{-3}) containing K$_2$S$_2$O$_8$ (0.2 mol dm^{-3}) and 2-methyl propan-2-ol (0.2 mol dm^{-3}) at 2 μs after the pulse at pH 9.5 (■), and the OH adduct spectrum of DMU at pH 10.4 at 85 μs after the pulse (▲) [43]. The absorbance is calculated for a typical dose rate of 10 Gy/pulse and a $G(\cdot OH)$ of 2.7×10^{-7} mol J^{-1}. Inset: decay trace of the intermediate obtained from the reaction of SO$_4^{\cdot-}$ at 425 nm.

the pH of the solution was 9.5, a considerable part of MU is in its deprotonated from. However, the base elimination reaction would be very similar to that from the neutral from. Therefore the final product (i.e. the oxyradical as shown in Scheme 4) would remain the same.

The transient absorption spectra obtained with DMU at pH 3 and at pH 9.5 are shown in Figs 7 and 8. The OH adduct spectra at these pH values are also shown in the same figures. Similar to MU, DMU has also two H-atoms bonded to N(1) and N(3) and hence it is expected that the deprotonation from these positions will be the most favored reaction of the radical cation of DMU. The transient spectrum at pH 3 is therefore attributed to the N-centered radical similar to MU (Scheme 5). However, at pH 9.5, the short-lived radical cation can react with OH$^-$ resulting an OH adduct. This possibility can be well understood from the close similarity of the spectrum with that of the OH adduct spectrum of DMU recorded at 85 μs after the pulse (Fig. 8). The OH adduct spectrum of DMU at 85 μs after the pulse is attributed to the oxy radical (at C(4)-O) which has been formed from the dehydration reaction of the initially formed C(6)-ylC(5)OH radical [43]. Therefore, the spectrum observed at pH 9.5 in the case of the reaction of SO$_4^{\cdot-}$ with DMU is assigned to the oxy radical as shown in Scheme 5. Similar to MU, DMU also has a pK value at 9.8. However,
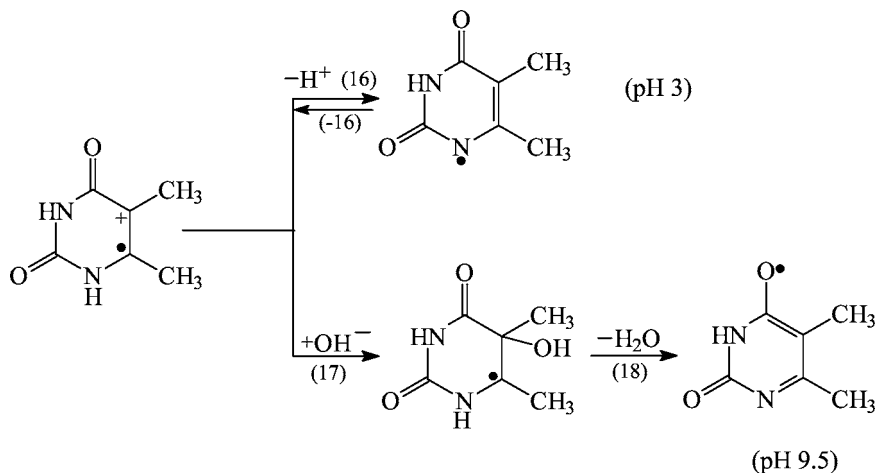
Scheme 5. Proposed mechanism of the reaction of sulphate radical with DMU.

the final radical product after the base elimination from the OH adduct would be the same.

CONCLUSIONS

The fate of the radical cation of DHMP, DMHP, MU and DMU resulting from the reaction of $SO_4^{\cdot-}$, is decided by the type of substituents and their position in the pyrimidine ring. Among the radical cations of these four pyrimidines, only the one from DMHP at acidic pH is found to be relatively stable. At basic pH all the radical cations of these compounds react with OH^- to form the corresponding OH adducts.

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